PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	Miao and Kay	EXAMINER:	Burkhart
APPLICATION NO.:	09/884,901	ART UNIT:	1633
FILED:	June 18, 2001	CONF. NO:	1704
FOR:	LIVER-SPECIFIC GENE EXPRESSION CASSETTES, AND METHODS OF USE		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration under 37 C.F.R. § 1.132

Sir:

I, Mark A. Kay, M.D., Ph.D., declare and affirm as follows:

1. I am an inventor of the subject matter described and claimed in U.S. Patent Application No. 09/884,901, filed June 18, 2001, titled "Liver-Specific Gene Expression Cassettes, and Methods of Use."

2. I am a professor in the departments of pediatrics and genetics at Stanford University and have over 15 years of research and experience in the technical area of U.S. Application No. 09/884,901 ("the application").

3. I have done extensive research and have published numerous peer-reviewed articles in the technical area of the application and, accordingly, have a strong understanding of the publications of my colleagues and peers, including the teachings provided in each reference cited by Examiner Michael D. Burkhart ("the examiner") during the examination of the application.

4. I have read and understand the office action mailed by the examiner on May 30, 2006, and am hereby submitting a statement that supports the patentability of the pending claims, along with the references that I have cited herein to further support this declaration.

5. I hereby submit that the pending claims recite a nucleic acid expression cassette capable of expressing human Factor IX, wherein the cassette is predominantly expressed in the mammalian liver of a post-natal subject, said cassette comprising:

(a) a hepatic locus control element consisting of SEQ ID NO:4 or SEQ ID NO:9;

(b) a heterologous hepatic promoter located 3' to the hepatic locus control element, said promoter consisting of a human α-1 antitrypsin promoter (SEQ ID NO:5);

(c) a Factor IX coding sequence located 3' to the hepatic promoter, said coding sequence comprising SEQ ID NO:2;

(d) a polyadenylation signal located 3' to the intron sequence, said polyadenylation signal consisting of SEQ ID NO:6; and

(e) an intron located 3' to the hepatic promoter and 5' to the polyadenylation signal, wherein said intron consists of SEQ ID NO:1,

wherein elements (a), (b), (c), (d) and (e) are operably linked to express the polypeptide encoded by the coding sequence.

6. First and foremost, I hereby submit that U.S. Patent No. 6,936,243 ("Snyder") is not a prior art reference to the pending claims of the application, since the reference is used against the application only for the construct that is taught in that patent, and among the inventors listed in Snyder, that construct is attributed soley to me as a product of my own work and not a product of the work of any the other inventors listed on the Snyder patent.

7. I hereby submit that, regardless of the construct in Snyder being attributable only to me among the list of inventors in Snyder, the pending claims were not obvious at the time the application was filed. As background, in the mid-1980s, Darnell's lab[1] showed that liver specific genes are not

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[1] Clayton D.F., Darnell J.E. Jr. Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. Mol. Cell. Biol. 1983 Sep;3(9):1552–1561; and

Isom L., Georgoff I., Salditt-Georgieff M., Darnell J.E. Jr. Persistence of liver-specific messenger RNA in cultured hepatocytes: different regulatory events for different genes. J. Cell. Biol. 1987

regulated correctly in primary hepatocyte cultures. There was no *a priori* expression profiles or explanations for this. In 1988 and 1991, Brinster and Palmiter[2] showed that introns substantially enhanced transgene expression in transgenic mice but not tissue culture cells. Brinster and Palmiter used a heterologous promoter and various transgenes, and they explained that the difference in the enhancement of transgene expression that was observed was due to the fact that the transgenic mice underwent a developmental alteration of the genes. Given the prediction, model, and reasoning provided by Brinster and Palmiter, those skilled in the art believed that the addition of an intron would not enhance transgene expression in the case of in vivo delivery of a transgene into post-natal animals.

8. Furthermore, Kurachi et al., J. Biol. Chem., Vol. 270 (10):5276 (1995) ("Kurachi"), cited against the application in the present action, established only that when using the endogenous Factor IX promoter to drive the Factor IX gene, the inclusion of a portion of the 1st intron enhanced Factor IX expression in tissue culture cells. I have shown in my previous publications, Kay et al., 1991, 1992 and 1993,[3] that exogenous expression cassettes are regulated differently in

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Dec;105(6 Pt 2):2877-85.

[2] Brinster R.L., Allen J.M., Behringer R.R., Gelinas R.E., Palmiter R.D. Introns increase transcriptional efficiency in transgenic mice. Proc. Natl. Acad. Sci. USA 1988 Feb.;85(3):836-40; and

Palmiter R.D., Sandgren E.P., Avarbock M.R., Allen D.D., Brinster R.L. Heterologous introns can enhance expression of transgenes in mice. Proc. Natl. Acad. Sci. USA 1991 Jan. 15;88(2):478-82.

[3] Kay, M. A., F. Graham, F. Leland, S. L. C. Woo. 1995. Therapeutic serum concentrations of human alpha 1-antitrypsin after adenoviral-mediated gene transfer into mouse hepatocytes. Hepatology 21:515-519;

Kay, M. A., P. Baley, S. Rothenberg, F. Leland, L. Fleming, K. Ponder, T. J. Liu, M. Finegold, G. Darlington, W. Pokorny, and S. L. C. Woo. 1992. Expression of human alpha-1-antitrypsin in dogs after autologous transplantation of retroviral transduced hepatocytes. Proc. Natl. Acad. Sci. USA 89:89-93; and

Kay, M. A., Q. T. Li, T. J. Liu, F. Leland, M. Finegold and S. L. C. Woo. 1992. Direct hepatic gene delivery in mice results in persistent expression of human alpha-1-antitrypsin in vivo.

cultured primary hepatocytes when compared to in vivo in a liver. The above findings would lead one of skill to believe, at the time the present application was filed, that there would be no way to predict with any reasonable expectation of success (1) which promoters would perform better under in vivo conditions based on in vitro studies, and (2) whether there would be any in vivo enhancement of expression using the intron of Kurachi.

9. Furthermore, a paper that I published with Miao in Molecular Therapy Vol. 1 (6):522(2000), cited by the examiner in the office action dated December 12, 2005,[4] demonstrated that an expression cassette of the present invention, having a combination of an intron and the heterologous promoter, resulted in almost no enhancement of transgene expression in cultured hepatocytes and hepatoma cell lines but unexpectedly increased transgene expression by nearly 100 fold in vivo when given to post-natal animals. Thus, in view of the state-of-the-art at the time the application was filed, the Kurachi paper was the only one at odds with the other results, and this is likely due to the fact that Kurachi used the Factor IX intron with the homologous Factor IX promoter.

10. I hereby submit that there is no significant influence on transgene expression in vivo in post-natal animals when the expression cassette is constructed without the untranslated region located 3' to the coding region and the intron.

11. Accordingly, based on the information available at the time the present application was filed, including the information discussed herein, one of skill (1) could not reasonably expect that the addition of an intron would enhance transgene expression in the case of in vivo delivery of a transgene into post-natal animals; (2) could not reasonably expect that any particular promoter would perform better under in vivo conditions based on in vitro studies; (3) could not reasonably expect that an expression cassette, such as the expression cassette of claim 1 having a combination of an intron and a heterologous promoter, would increase transgene expression nearly 100-fold in vivo when given to

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Human Gene Therapy 3:641-647.

[4] Note that this reference is not prior art, because it has a publication date of no earlier than June 26, 2000. See the Response filed March 8, 2006.

post-natal animals, particularly since there was almost no enhancement with in vitro transgene expression in cultured hepatocytes and a hepatoma cell line.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the Unites States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

11/30/06
Date

Mark A. Kay, M.D., Ph.D.